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Abstract. Diet supplementation with fish oil (FO) inhibits the saturation of *trans*-18:1 to 18:0 in the rumen, increasing the accumulation of *t*11-18:1 and, consequently, the concentration of the potentially health-promoting *c*9*t*11-conjugated linoleic acid (CLA) in milk. However, this feeding strategy also induces milk fat depression (MFD), which has been associated with a shortage of 18:0 for mammary *c*9-18:1 synthesis and its possible impact on the maintenance of milk fat fluidity. Thus, with the aim of studying whether FO-induced MFD can be alleviated by increased availability of 18:0 for mammary Δ^9-desaturation (i.e., for its conversion to *c*9-18:1), an experiment was performed in dairy ewes. The trial followed a 3×3 Latin square design (4 ewes/group) with 3 periods of 4 weeks each and 3 experimental diets: non-supplemented, supplemented with 2% FO and supplemented with 2% FO plus 2% 18:0. Milk production and composition were analyzed on the last 3 days of each period. At the end of the experiment, the digestibility of supplemental 18:0 was measured using 6 lactating sheep. Supplemented diets had no significant effect on milk yield but, compared with the control, both of them reduced milk fat content in a similar proportion (-20%), which suggests that the addition of 18:0 to the diet does not alleviate FO-induced MFD. Since this result cannot be fully explained by the relatively low digestibility coefficient of the 18:0, further research would be required to elucidate if the lack of response to this fatty acid was attributable to a low mammary uptake or to other factors.

Keywords. Sheep – Lipid – Marine oil – Milk fat depression.

Effets de la supplémentation en 18:0 sur la teneur de matières grasses du lait chez des brebis laitières alimentées avec un régime riche en huile du poisson

Résumé. *La supplémentation du régime avec de l'huile de poisson (FO) peut améliorer la composition en acides gras du lait mais cause une diminution du taux butyreux (connu sous le nom de «milk fat depression» ; MFD), qui a été associé à une faible disponibilité de 18 : 0 pour la synthèse mammaire de* c*9-18 : 1 et sa répercussion potentielle sur la régulation de la fluidité des matières grasses du lait. Ainsi, dans l'objectif d'étudier si la MFD causée par FO peut être palliée par une disponibilité accrue de 18 : 0 pour sa* Δ^9*-désaturation mammaire en* c*9-18 : 1, un essai a été réalisé sur des brebis laitières selon un carré latin 3×3 (4 brebis/lot). Les animaux ont reçu 3 régimes expérimentaux au cours de 3 périodes de 4 semaines : un régime sans supplémentation lipidique ou supplémenté avec FO (2%) ou avec FO (2%) plus 18 : 0 (2%). Des contrôles de production laitière et composition du lait ont été réalisés à la fin de chacune des périodes. La digestibilité du supplément de 18 : 0 a été mesurée sur 6 brebis laitières en fin d'expérimentation. Les suppléments lipidiques n'ont pas eu d'effet sur l'ingestion ou la production laitière mais, comparés au témoin, ils ont induit des chutes similaires du taux butyreux du lait (-20%), ce qui suggère que le supplément de 18 : 0 n'atténue pas la MFD induite par FO. Toutefois, ce résultat n'est pas entièrement expliqué par une relativement faible digestibilité du 18 : 0 et une recherche plus approfondie serait nécessaire pour déterminer si l'absence de réponse à cet acide gras est due à une faible captation mammaire ou à d'autres facteurs.*

Mots-clés. *Mouton – Lipide – Huile marine – Chute du taux butyreux.*

I – Introduction

Development of feeding strategies to modulate the nutritional quality of sheep milk and improve its added value cannot be accompanied by detrimental effects on animal performance, since this would prevent their application under practical farm conditions.

Supplementation of dairy ewe diet with fish oil or marine microalgae inhibits the saturation of *trans*-18:1 to 18:0 in the rumen, increasing the accumulation of *t*11-18:1 and, consequently, the concentration of the potentially health-promoting *c*9*t*11-conjugated linoleic acid (CLA) in milk (Toral *et al.*, 2010a; Bichi *et al.*, 2013). Furthermore, marine lipids are rich in long-chain n-3 polyunsaturated fatty acids that can be transferred into milk fat (Lock and Bauman, 2004). However, this feeding strategy induces milk fat depression (MFD; Shingfield *et al.*, 2010; Toral *et al.*, 2010a), which has been associated with a shortage of 18:0 for mammary uptake (due to the mentioned inhibition of the last step of ruminal biohydrogenation). This shortage could limit the mammary endogenous synthesis of *c*9-18:1, a fatty acid (FA) with a low melting point that contribute to maintain milk fat melting point below body temperature and ensure milk fat fluidity and subsequent milk fat secretion (Gama *et al.*, 2008; Shingfield *et al.*, 2010; Bichi *et al.*, 2013).

On this basis, this study was conducted to test the hypothesis that supplemental 18:0 would alleviate fish oil-induced MFD in dairy ewes.

II – Material and methods

Twelve lactating Assaf ewes (78.5 ± 2.43 kg of body weight; 32 ± 1.5 days in lactation milk at the beginning of the assay) were allocated to one of 3 groups (n = 4) balanced for milk production and composition, body weight, days in milk, and parity and used in a 3 × 3 Latin square design to test the effects of 3 dietary treatments during 3 experimental periods of 28 days each. Diets consisted of a total mixed ration (TMR) containing no additional lipid (control) or 2% DM of fish oil (Afampes 121 DHA; Afamsa, Mos, Spain) alone (FO) or in combination (FOSA) with 2% DM of 18:0 (Edenor C18 98-100; Oleo Solutions, York, UK). The TMR was formulated (g/kg) from dehydrated alfalfa hay (400), whole maize (180) and barley (130) grains, soybean meal (150), beet pulp (70), molasses (50), and minerals and vitamins (20). All ewes were fed the control diet during 3 weeks of adaptation before starting the study. The TMR was offered *ad libitum* twice daily, at 9:30 and 18:30 h. Ewes had continuous access to clean drinking water and were milked twice daily at approx. 9 and 18 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain).

Dry matter intake was recorded during the last week of each period and samples of offered diets were analyzed for chemical composition (Bichi *et al.*, 2013). Milk yield was recorded on days 25, 26, and 27 of each experimental period. With the same frequency, individual milk samples were collected and composited according to morning and evening milk yield, preserved with bronopol and stored at 4ºC until analyzed for fat, protein, and lactose by infrared spectrophotometry (ISO 9622:1999).

At the end of the experiment, 3 ewes on FOSA and 3 on FO (to determine the concentration of 18:0 when this fatty acid is not added to the diet) were housed in individual metabolic cages to examine the *in vivo* digestibility of the 18:0 supplement. After 2 days of adaptation to the cages, DM intake was recorded and feces were weighed daily over 5 consecutive days. Individual samples of feeds offered and refused and of feces were collected and stored at -30ºC until analyzed for DM and lipid composition. Following a change-over design, diets received by each lot were then switched and offered for 21 more days. On the last 7 days of the second period, ewes were housed in metabolic cages and the same measurements and sampling procedures were conducted. Fatty acid methyl esters (FAME) of lipid in freeze-dried samples of diets and orts were prepared in a 1-step extraction-transesterification procedure (Shingfield *et al.*, 2003). Lipid in 200 mg of

freeze-dried feces was extracted and then converted to FAME by sequential base-acid catalyzed transesterification (Toral *et al.*, 2010b). In both cases, *c*12-13:1 (Larodan Fine Chemicals AB, Malmö, Sweden) was used as internal standard. Quantification of 18:0 was performed on a gas chromatograph (Agilent 7890A GC System, Santa Clara, USA) equipped with a fused silica capillary column (100 m × 0.25 mm i.d.; CP-SIL 88, Varian Ibérica S.A., Madrid, Spain), using a temperature gradient program (Shingfield *et al.*, 2003).

Animal performance and milk composition data were subjected to ANOVA for repeated measures using the MIXED procedure of the SAS software package (version 9.3, SAS Institute Inc., Cary, NC, USA). The statistical model included the fixed effects of treatment, experimental period, and day as a repeated measure. The lot nested within the treatment was used as the error term to contrast the effect of experimental diet. Means were separated using the "pdiff" option of the "lsmeans" statement.

III – Results and discussion

The basal TMR (to which the supplements were added) contained, per kg of DM, 911 g of organic matter, 185 g of crude protein, and 246 g of neutral detergent fiber. The ether extract concentration per kg of DM was 23 g for the control, 43 g for FO, and 61 g for FOSA diet.

As shown in Table 1, diet supplementation with fish oil had no effect on milk yield ($P>0.10$), in agreement with previous studies in dairy ewes receiving marine lipids (Toral *et al.*, 2010a; Bichi *et al.*, 2013), despite FO diet tended to decrease DM intake relative to the control ($P<0.10$). The slight reduction in milk protein content after the inclusion of lipids in the diet (on average, -7%; $P<0.001$) was within the range commonly observed for sheep fed oil supplements (Pulina *et al.*, 2006; Toral *et al.*, 2010a).

Table 1. Dry matter intake, and milk yield and composition in dairy ewes fed a total mixed ration without lipid supplementation (Control) or supplemented with 2% DM of fish oil alone (FO) or in combination with 2% of 18:0 (FOSA)

		Diet			s.e.d.	*P*-value
		Control	FO	FOSA		
Dry matter intake (g/d)		3369	2974	3249	105.8	0.088
Yield (g/d)	Milk	3157	3093	3212	86.8	0.407
	Fat	168[a]	134[b]	136[b]	4.4	<0.001
	Protein	171[a]	157[b]	163[ab]	3.9	0.008
	Lactose	159	158	162	5.2	0.732
Composition (%)	Fat	5.35[a]	4.32[b]	4.22[b]	0.094	<0.001
	Protein	5.44[a]	5.09[b]	5.07[b]	0.051	<0.001
	Lactose	5.02	5.09	5.03	0.038	0.186

s.e.d. = standard error of the difference.

[a-b] Different superscripts within a row indicate differences at $P<0.05$.

As expected, FO diet caused MFD in ewes, with significant reduction in milk fat concentration and yield compared with the control ($P<0.001$; Table 1). With regard to the mechanisms involved in this response, it has been suggested that the shortage of 18:0 for mammary *c*9-18:1 synthesis might detrimentally affect milk fat melting point, exceeding the capacity of the mammary epithelial cell to maintain an adequate milk fat fluidity, and thereby decreasing the rate of fat secretion (Gama *et al.*, 2008; Shingfield *et al.*, 2010; Toral *et al.*, 2010a). However, surprisingly, both FO and FOSA decreased milk fat content and yield in a similar proportion (-20% relative to the control; $P<0.001$), which might point to a major contribution of other FA or to other mechanisms explaining FO-induced MFD.

Under normal feeding conditions, the digestibility of 18:0 averages 75% (Loften *et al*., 2014). Nevertheless, it has been shown to decrease at high 18:0 flows into the duodenum (Glasser *et al.*, 2008; Loften *et al.*, 2014), which may explain the relatively low digestibility coefficient of supplemental 18:0 (on average, 50%). In any event, this would not, in isolation, appear to fully account for its lack of effect in dairy ewes. In this regard, the amount of additional 18:0 absorbed in the digestive tract of sheep fed FOSA was calculated to represent approx. 32 g/d, which largely exceeds the estimated decrease in 18:0 during marine lipid-induced MFD in high-production Assaf ewes (Toral *et al.*, 2010a; Bichi *et al.*, 2013). Thus, the reasons underlying the absence of response to the 18:0 supplement are not obvious, and further research would be required to elucidate if this was attributable to a low mammary uptake (Enjalbert *et al.*, 1998) or to others factors that might be counteracting the potential effects of increased 18:0 availability.

IV – Conclusion

Similar reductions in milk fat content and yield with FO and FOSA diets demonstrate that supplemental 18:0 does not alleviate marine lipid-induced MFD in dairy ewes. This result cannot be fully explained by the relatively low digestibility coefficient of the 18:0, challenging the hypothesis of decreased 18:0 availability as a mechanism to explain this type of MFD in sheep.

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